Alloplex Biotherapeutics

SCIENTIFIC MONOGRAPH

Review of NK and NKT-like Cell Biology

Immunosurveillance of Cellular Stress and Regulation of Inflammation

Frank Borriello, MD, PhD

Chief Executive Officer, Alloplex Biotherapeutics Inc.

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PART I

Foundations

1 Introduction: One Lineage, Two Programs

Natural killer (NK) cells and natural killer T (NKT)-like cells are most often introduced as the immune system's rapid-response cytotoxic arm — innate lymphocytes that produce IFN-γ within hours, kill virus-infected and transformed cells, and amplify downstream inflammation through TNF, GM-CSF, and chemokine secretion. This effector portrait is accurate but incomplete. Over the past two decades, a parallel body of evidence has established that the same two cell lineages — and frequently the same individual cells — are also potent attenuators of inflammation. They produce IL-10 and TGF-β, kill activated autologous T cells and dendritic cells, polarize macrophages toward an M2 resolution phenotype, induce regulatory T cells (Tregs), edit the senescent and fibrogenic compartments of injured tissues, and cross-regulate one another to terminate inflammatory cascades.

The central question is not whether NK and NKT-like cells can be regulatory — that is now beyond dispute — but how a single cell type executes opposite immunological programs depending on context. The answer is not, as is sometimes implied, that there are two cleanly separable subsets, one inflammatory and one regulatory. The reality is more interesting: while certain subsets are biased toward one polarity (CD56^{bright} NK cells and NKT10/type II NKT cells lean regulatory; CD56^{dim} NK cells and Th1-polarized iNKT1 cells lean inflammatory), individual cells exhibit substantial functional plasticity, and the polarity that dominates *in vivo* is determined by the integration of receptor signals, cytokine milieu, tissue microenvironment, target-cell phenotype, and the temporal phase of the response.

A second organizing theme runs through this review and connects the regulatory biology to a therapeutic application that has only recently come into focus. The recognition system these cells use is not antigen-specific; it is a stress-detection system. NK and NKT-like cells read a coordinated surface signature induced by cellular distress — DNA damage, endoplasmic-reticulum stress, integrated stress-response activation, loss of membrane asymmetry — and they read it through several receptors simultaneously. That same signature is displayed by tumor cells, virally infected cells, and senescent cells. The immunosurveillance of senescence, which comprises an integral part of somatic homeostasis, is therefore not a separate biological program bolted onto NK biology; it is the same recognition machinery applied to different cell targets. In the case of senescent cells this connects the immune system to controlling senescent cells and thus regulating age-related inflammation, fibrosis, and organ dysfunction. The senolytic function and tumor immunosurveillance are two expressions of a single system.

The review is organized in six parts.

- **Part I** establishes the cellular taxonomy.
- **Part II** details the recognition system — the activating and inhibitory receptors, the stress programs that induce their ligands, and the cytolytic machinery that executes the response.

- **Part III** covers the mechanisms by which NK and NKT-like cells attenuate inflammation.
- **Part IV** covers immunosurveillance of cellular senescence, the escape mechanisms that defeat it, and the *in vivo* evidence for therapeutic senescent-cell clearance.
- **Part V** synthesizes the dual-role paradox and its therapeutic implications.
- **Part VI** addresses relevance to SUPLEXA™ development strategy.

2 Cellular Taxonomy and Subset Biology

2.1 NK cell subsets

Human NK cells are conventionally divided into two major subsets based on CD56 surface density. $CD56^{dim}$ $CD16^{+}$ NK cells represent approximately 90% of circulating NK cells and are predominantly cytotoxic, expressing high levels of perforin, granzymes, and the Fcγ receptor CD16 that mediates antibody-dependent cellular cytotoxicity (ADCC). $CD56^{bright}$ $CD16^{-}$/dim NK cells represent approximately 10% of circulating NK cells but are enriched in secondary lymphoid tissues; they are the principal NK source of immunoregulatory cytokines (IFN-γ, TNF, GM-CSF, IL-10, IL-13) and are the subset most consistently associated with regulatory function in autoimmune disease [1–3]. The functional dichotomy is real but graded rather than absolute: $CD56^{bright}$ cells acquire cytotoxic competence under sustained IL-15 or IL-12/IL-18 exposure, and $CD56^{dim}$ cells produce substantial cytokine on strong activating-receptor engagement [4,5].

2.2 Tissue-resident NK populations

Tissue-resident NK (trNK) populations expand this picture considerably. Liver-resident, decidual, lung-resident, and uterine NK cells each display distinct transcriptional programs, receptor repertoires, and cytokine outputs imprinted by their tissue of residence. Decidual and uterine NK cells are functionally biased toward angiogenic and tolerogenic outputs (VEGF, PIGF, IL-10) rather than cytotoxicity — a tissue-imprinted regulatory phenotype critical for trophoblast invasion and pregnancy maintenance. Tissue imprinting is not fixed: the obese liver environment converts resident NK cells to a less cytotoxic, ILC1-like phenotype [6], demonstrating that the local stromal milieu can rewrite functional identity *in situ*.

This plasticity is central to the paradox developed in Part V, and it is the reason that assessing an NK population by its circulating phenotype alone can be misleading about what it will do once it reaches a tissue and is acted upon by the local environment. It also reframes the underlying taxonomic question: whether trNK cells constitute a genuinely distinct population, or are simply NK cells that have responded and adapted to the local environment. The available data do not cleanly resolve this, and for therapeutic purposes the distinction may matter less than the observation itself — that the tissue, not the lineage label, determines a substantial part of the functional profile.

2.3 NKT-like cell subsets

NKT-like cells are a heterogeneous population, some of which are CD1d-restricted T cells and recognize lipid antigens. The two principal divisions among CD1d-restricted NKT cells are type I (invariant; iNKT) and type II (diverse-TCR; sometimes called dNKT). Type I iNKT cells express a semi-invariant TCR (Vα24-Jα18/Vβ11 in humans; Vα14-Jα18/Vβ8 in mice) and respond strongly to α-galactosylceramide (αGalCer). Type II NKT cells have diverse TCRs and recognize self lipids including sulfatide, lysophosphatidylcholine, and β-glucosylceramide [7,8].

Within the iNKT compartment, functional polarization mirrors the Th1/Th2/Th17 paradigm: iNKT1 cells (T-bet$^+$) produce IFN-γ; iNKT2 cells (GATA3$^+$) produce IL-4 and IL-13; iNKT17 cells (RORγt$^+$) produce IL-17; iNKT-FH cells provide B cell help; and a regulatory subset, NKT10 cells (E4BP4$^+$), produces IL-10 and lacks the canonical iNKT transcription factor PLZF [9,10]. Type II NKT cells and NKT10 cells are predominantly regulatory and consistently antagonize type I iNKT-driven inflammation, establishing a built-in cross-regulatory axis within the compartment itself (Section 8.2). Thymic development of these unconventional lineages, and of the γδ lineage discussed next, has been reviewed comprehensively [11].

A note on nomenclature. Formal assignment of a cell to the NKT lineage requires demonstration of CD1d restriction and, for type I cells, of the invariant TCR — parameters that are not routinely measured in phenotypic characterization panels. Throughout this review, "NKT-like" denotes the broader population identified by surface phenotype, while "iNKT", "type II NKT", and "NKT10" are used only where the cited work established the defining parameters. This distinction matters for any product whose composition is defined by flow or mass cytometry rather than by tetramer and restriction analysis.

2.4 Vδ1 γδ T cells — the third innate-lineage stress sensor

A complete account of innate lymphocyte stress surveillance must include γδ T cells, which share the germline-encoded activating-receptor repertoire of NK cells while retaining a T-cell receptor. The human γδ compartment divides functionally along the Vδ1/Vδ2 axis. Vγ2Vδ9 cells are the dominant circulating subset, phosphoantigen-responsive, BTN3A1/BTN2A1-dependent, and biased toward acute inflammatory output. Vδ1 cells are predominantly tissue-resident, BTNL3/BTNL8-responsive at epithelial surfaces, and biased toward immunoregulation and stress surveillance rather than acute inflammation. Critically for the subject of this review, Vδ1 cells use NKG2D as a major activating receptor and recognize MIC and ULBP family ligands directly [12,13]. Hayday's framing of the γδ compartment as a "lymphoid stress-surveillance response" [12] is the conceptual bridge between the γδ literature and the NK literature: both lineages detect the same induced-self ligands through the same receptors, and both are recruited by the same tissue distress signals. Vδ1 cells accumulate in aged human tissues and kill senescent fibroblasts in an NKG2D-dependent manner (Section 11).

PART II

The Recognition System

3 Activating Receptors

The activating receptor repertoire of innate lymphocytes is germline-encoded and non-clonal. Rather than recognizing a specific antigen, each receptor detects a molecular consequence of cellular distress. The receptors are functionally non-redundant in the sense that they read different upstream stress programs, and functionally synergistic in the sense that no single receptor other than CD16 is sufficient to trigger cytotoxicity in a resting cell [106*]. Both properties matter, and both are developed below. The proximal signaling architecture is shared: most activating receptors couple to ITAM-bearing adaptors (DAP12, FcRγ, CD3ζ) or, in the case of NKG2D in humans, to the YINM-motif adaptor DAP10, converging on Syk/ZAP-70 or PI3K and Vav1 to drive granule polarization and cytokine transcription [107*].

3.1 NKG2D and the DNA damage response

NKG2D (KLRK1) is the best-characterized activating receptor in this system and the one with the deepest literature. It recognizes the MHC class I-like stress ligands MICA and MICB and the ULBP/RAET1 family (ULBP1–6) in humans, with orthologous RAE-1 (α–ε), H60 (a–c), and MULT1 in mice [14–16]. NKG2D was cloned and characterized as an activating receptor on NK and $CD8^+$ T cells with MICA as its first identified ligand [14]; the ULBP family followed [15]. The conceptual framework that Raulet established [16,17] remains the correct one: NKG2D is a germline-encoded stress sensor expressed by several lymphoid lineages that allows immune cells to recognize cells under duress — DNA damage, viral infection, oncogenic transformation — without requiring MHC-restricted antigen presentation, and is hence part of the innate immune system.

The mechanistic anchor for everything that follows is Gasser and colleagues' demonstration that the DNA damage response, specifically ATM/ATR signaling, is the pathway that induces NKG2D ligand expression [19]. The same DNA damage signaling that drives p53/p21/p16-mediated cell-cycle arrest drives NKG2D-ligand surface display. The regulation of both NKG2D and DNAM-1 ligands by the DNA damage response across normal, infected, and cancer cells has been reviewed comprehensively [20]. This single fact — that the pathway which creates senescence is the pathway that flags it for immune recognition — is what makes senescent cells intrinsic NK targets rather than incidental ones.

NKG2D possesses no intracellular signaling domain, hence signaling proceeds through the adaptor protein DAP10 in humans, which recruits PI3K and Grb2/Vav1 and drives cytotoxic granule polarization, target killing, and IFN-γ production [18]. On $CD8^+$ T cells, NKG2D functions as a costimulatory receptor that lowers the TCR activation threshold against ligand-positive targets, and IL-15 induces NKG2D on CD28-negative effector CTL in the tissue microenvironment [29]. On Vδ1 and Vδ2 γδ T cells, NKG2D engagement is directly activating [13].

The principal evasion mechanism against this axis is ligand shedding. ADAM-family proteases release soluble MICA/MICB from the target surface, and the resulting soluble decoys down-modulate NKG2D expression on the effector and impair T-cell activation [21]. This is a well-documented tumor escape route, and is one of the routes by which long-persisting senescent cells become refractory to clearance.

3.2 DNAM-1 and the nectin axis

DNAM-1 (CD226) binds the nectin and nectin-like molecules CD155 (PVR/Necl-5) and CD112 (PVRL2/nectin-2) [22]. Both ligands are co-induced with the NKG2D ligands by the same ATM/ATR DNA damage response, which is why the two axes are usually engaged together rather than independently. DNAM-1 is constitutively expressed on essentially all NK cells and on most T cells, and it functions principally through adhesion strengthening and by lowering the threshold for degranulation triggered by other receptors, rather than as a stand-alone trigger [106*].

The axis is complicated by shared-ligand competition. CD155 is bound not only by activating DNAM-1 but by the inhibitory receptors TIGIT and CD96, and TIGIT binds CD155 with higher affinity than DNAM-1 does [23,117*]. Net signaling through the nectin axis therefore depends on the relative surface density of DNAM-1 versus TIGIT on the effector, not on ligand abundance on the target. An effector with high TIGIT is outcompeted for CD155 by its own inhibitory receptor and extracts little activating signal even when the target displays abundant ligand. Because TIGIT expression rises on lymphocytes in aging and chronic inflammation as part of immunosenescence, this competition is a structural reason why endogenous surveillance of nectin-ligand-positive targets degrades with age.

3.3 The natural cytotoxicity receptors: NKp30, NKp44, NKp46

The three natural cytotoxicity receptors (NCRs) — NKp46 (NCR1, CD335), NKp44 (NCR2, CD336), and NKp30 (NCR3, CD337) — were cloned by the Moretta group in the late 1990s and are the major activating receptors for non-MHC-restricted recognition of tumor and stressed cells in humans [108*,109*]. NKp46 and NKp30 are constitutively expressed on resting NK cells and signal through CD3ζ and FcRγ; NKp44 is absent from resting cells and is induced by IL-2 or IL-15, signaling through DAP12. Their ligands were, for two decades, substantially less well defined than the NKG2D ligands, and this gap has closed only recently.

NKp46 (NCR1, CD335). NKp46 is the most lineage-restricted NCR and the closest thing the field has to a pan-NK marker, conserved across mammalian species. Confirmed binding partners long included viral hemagglutinins (influenza HA, parainfluenza HN), heparan sulfate proteoglycans, complement factor P, and an unidentified ligand on activated hepatic stellate cells [24,57]. The major recent advance is the identification of ecto-calreticulin — the endoplasmic-reticulum chaperone calreticulin externalized to the cell surface under ER stress — as an activating NKp46 ligand, with explicit demonstration of ecto-calreticulin display on stressed and senescent cells and NKp46-dependent killing of those targets [25]. This finding is mechanistically important because it is orthogonal to the DNA damage axis: NKG2D and DNAM-1 ligands are induced by ATM/ATR, whereas ecto-calreticulin externalization is driven by the unfolded protein response. Both pathways are simultaneously active in senescent cells, so a target that escapes NKG2D recognition through MICA/MICB shedding may still

be visible through NKp46, and vice versa. NKp46 engagement drives both granule exocytosis and IFN-γ production, and NKp46-dependent killing of activated hepatic stellate cells is one of the clearest *in vivo* demonstrations of an NCR constraining fibrosis [57].

NKp30 (NCR3, CD337). NKp30 is the NCR most directly implicated in the cross-talk between NK cells and dendritic cells: it is the receptor through which activated NK cells recognize and edit immature DCs [48], linking it to the quality-control function described in Section 7.2. Its best-defined cellular ligand is the B7-family member B7-H6 [26]. B7-H6 is induced by the integrated stress response on tumor cells, virally infected cells, and inflammatory cells; expression on healthy tissue is essentially undetectable. It is found on roughly 20% of human tumor cell lines including melanoma, carcinomas, T- and B-cell lymphomas, and myeloid leukemias, as well as on primary patient tumor and marrow cells. NKp30 has three splice isoforms (NKp30a, b, and c) whose relative expression determines whether engagement produces an immunostimulatory or immunosuppressive output, which is one reason NKp30 responses vary between donors. Direct B7-H6 expression on senescent cells has not been formally documented, and this remains one of the more tractable open questions in the field: the integrated stress response is active in senescence, so the prediction is straightforward and testable.

NKp44 (NCR2, CD336). NKp44 is the only NCR that is not constitutively expressed; its induction by IL-2 and IL-15 makes it a marker of cytokine-activated rather than resting NK cells, and therefore a receptor whose presence on any *ex vivo* expanded product is an expected consequence of the culture conditions. Its best-characterized ligand is PDGF-DD, a secreted growth factor produced by many tumors and dysregulated in fibrotic tissue including liver and lung [27]. NKp44 engagement of PDGF-DD does not directly trigger cytolysis; it drives IFN-γ and TNF-α secretion that induces tumor-cell growth arrest and amplifies local inflammatory signaling. NKp44 is unusual in also having a documented inhibitory ligand, PCNA, when the latter is aberrantly surface-expressed, so the net output of this axis is target-dependent [109*]. The relevance of NKp44 to senescence is indirect but real, because the tissues in which senescent cells accumulate are precisely the fibrotic tissues in which PDGF-DD is dysregulated.

3.4 Coactivating receptors: 2B4, CD2, and CD16

2B4 (CD244). 2B4 is a member of the SLAM family and binds CD48, which is broadly expressed on hematopoietic cells; the interaction is therefore relevant principally to hematopoietic targets and to NK–NK and NK–T interactions rather than to solid-tissue targets. Signaling proceeds through SAP (SH2D1A) and related adaptors, and the direction of the output depends on adaptor availability: with adequate SAP, 2B4 is activating; in SAP deficiency, as in X-linked lymphoproliferative disease, the same receptor becomes inhibitory [110*,111*]. Its function in normal NK cells is canonically coactivating rather than independently activating. Bryceson and Long's systematic pairwise analysis established that on resting human NK cells no single activating receptor other than CD16 triggers natural cytotoxicity alone, and that specific pairs — NKG2D with 2B4, DNAM-1 with 2B4, NKG2D with DNAM-1 — synergize to cross the degranulation threshold [28]. This is the single most important quantitative principle in the field and is developed further in Section 4.4.

CD2 and CD58. CD2 binds CD58 (LFA-3) and functions both as an adhesion stabilizer of the immunological synapse and as a costimulatory signal that lowers the activation threshold for cytotoxicity [112*]. CD2 is expressed on essentially all NK cells, NKT-like cells, and γδ T cells

including the Vδ1 subset. Because CD2 and CD58 are counter-receptors expressed on overlapping populations, the interaction operates both in trans, between effector and target, and homotypically between effectors — the latter producing the cell clustering characteristic of activated lymphocyte cultures. The avidity of the interaction is acutely regulated by activation state: lymphocyte activation increases CD2 surface number roughly 1.5-fold and increases the two-dimensional affinity of CD2 for CD58 roughly 2.5-fold, with reduced lateral mobility of ligated CD2, together substantially enhancing adhesion in the peak-activation state [36]. The relevance of CD58 as an induced ligand on senescent cells is developed in Section 5.4.

CD16 (FcγRIIIA). CD16 is the exception to the synergy rule. Engagement by antibody-opsonized target is sufficient on its own to trigger degranulation in a resting NK cell [28]. This is the molecular basis of ADCC and of every therapeutic-antibody combination strategy that relies on NK effector function [113*]. Two practical features follow. First, CD16 is shed from the NK surface by ADAM17 following activation, which limits serial killing and is the rationale for ADAM17 inhibition and for non-cleavable CD16 variants in engineered products. Second, the FCGR3A V158F polymorphism determines IgG1 Fc affinity and is a well-documented determinant of clinical response to rituximab and trastuzumab, making donor genotype a real source of inter-individual variability in antibody-combination settings.

3.5 TIM-3: lineage-divergent function

TIM-3 (HAVCR2) is the receptor most frequently misread in this system, because its function is inverted between lineages. On exhausted $CD8^+$ T cells TIM-3 is an inhibitory checkpoint, and this is the basis for the clinical TIM-3 antagonists in development. On NK cells the picture is different. Human NK cells transcribe the highest amounts of TIM-3 among all lymphocytes, TIM-3 is expressed on essentially all mature $CD56^{dim}$ $CD16^+$ NK cells, and TIM-3-high NK cells are fully responsive with respect to both cytokine production and cytotoxicity [32]. The complementary mechanistic finding is that TIM-3 functions on NK cells as an activating coreceptor for IFN-γ production: TIM-3-positive NK cells engaged by galectin-9 on tumor targets, including primary AML blasts that endogenously express galectin-9, produce significantly enhanced IFN-γ, and cytokine priming with IL-12, IL-15, or IL-18 amplifies this further [33].

TIM-3 has at least four characterized ligands: phosphatidylserine, externalized on apoptotic, stressed, and senescent cells [34,35]; galectin-9, induced on many tumors and on marrow stroma; CEACAM-1, on activated T cells and many epithelial tumors; and HMGB1, a DAMP released by damaged cells. Phosphatidylserine is the mechanistically important one for stress surveillance, and it connects TIM-3 to a function beyond cytotoxicity: TIM-3 mediates phagocytosis of apoptotic cells and cross-presentation of the antigen they carry [34]. An innate lymphocyte expressing TIM-3 therefore has, in principle, both a recognition channel for the phosphatidylserine-exposing target and a route to antigen-presenting-cell-like function against it.

3.6 Death-receptor ligands: TRAIL

TRAIL (TNFSF10) is unusual in this catalogue because the effector carries the ligand rather than the receptor. TRAIL was identified as a TNF-family member that induces apoptosis [30] and engages the pro-apoptotic death receptors DR4 (TRAIL-R1) and DR5 (TRAIL-R2) on the target, with DcR1 and DcR2

acting as decoy antagonists that titrate net susceptibility [31]. CD56^{bright} NK cells and liver-resident NK cells use TRAIL prominently. As Section 6 develops, decoy-receptor induction on senescent cells is one reason the death-receptor route is not the dominant senolytic mechanism.

4 Inhibitory Receptors and the Integration Rule

4.1 KIR and missing-self

The killer-cell immunoglobulin-like receptors (KIRs) bind HLA-A, -B, and -C allotypes and deliver ITIM-mediated inhibition. The missing-self principle follows directly [114*]: a target that has down-regulated class I to evade CD8$^+$ T-cell recognition loses KIR-mediated protection and becomes an NK target. The KIR locus is among the most polymorphic in the human genome and segregates independently of HLA, so KIR–HLA pairing varies between individuals and shapes both susceptibility to infection and outcome after allogeneic transplantation [115*]. NK-cell licensing during development calibrates responsiveness to self-MHC, so that unlicensed cells are hyporesponsive and licensed cells are potently triggered by MHC-low targets.

4.2 NKG2A and HLA-E

The CD94/NKG2A heterodimer binds the non-classical MHC molecule HLA-E, which presents leader peptides derived from classical class I molecules — a design that makes HLA-E surface density a proxy report on the integrity of the whole class I pathway. NKG2A is the dominant inhibitory receptor on CD56^{bright} NK cells and on terminally differentiated CD8$^+$ T cells, and NKG2A engagement is sufficient to override strong activating input. It is, as Sections 7.1 and 12.1 show, the axis that governs both the safety of NK-mediated regulation of activated T cells [43] and the escape of senescent cells from clearance [81]. The axis is clinically actionable: monalizumab, a humanized anti-NKG2A antibody, was developed to release this brake, and its clinical development provides the most direct evidence that the axis is rate-limiting in humans [116*].

4.3 TIGIT, CD96, PD-1, and CTLA-4

TIGIT and CD96 compete with DNAM-1 for CD155 and CD112 as described in Section 3.2, and TIGIT additionally delivers direct ITIM-mediated inhibition and can act in cis to disrupt DNAM-1 homodimerization [117*]. PD-1 binds PD-L1 and PD-L2; CTLA-4 binds CD80 and CD86. Both are canonical checkpoints of T-cell exhaustion, and both are variably expressed on NK cells depending on activation history and tissue context. The practical consequence is that the inhibitory repertoire of a given effector population is not fixed by lineage; it is a phenotype that must be measured, and it is the primary determinant of whether that population will function against targets that have deployed checkpoint-based escape.

4.4 Signal integration and the coactivation-synergy rule

NK and NKT-like cell function is the integral of activating and inhibitory receptor engagement, not the response to any single receptor [106*]. Three quantitative features of this integration deserve emphasis because they are frequently elided.

4.4.1 *Synergy is required*

On resting NK cells, no activating receptor other than CD16 triggers natural cytotoxicity when engaged alone; specific coactivating pairs are required to cross the degranulation threshold [28]. The requirement is not merely additive but reflects distinct and complementary proximal signals — for example, 2B4 and NKG2D contribute differently to Vav1 phosphorylation and to actin remodeling at the synapse, and neither alone is sufficient. This matters directly for senescent-cell targeting, because senescent cells display intermediate rather than maximal ligand densities on any single axis. It is the coincidence of several intermediate signals, not the magnitude of one, that crosses the threshold — which is also why a multi-receptor effector and a single-receptor engineered effector are not interchangeable against this target class.

4.4.2 *Inhibition is dominant, not additive*

A strong NKG2A or KIR signal overrides substantial activating input rather than subtracting from it. Inhibitory ITIM signaling recruits SHP-1 and SHP-2, which dephosphorylate Vav1 upstream of the point at which activating signals converge, so the block is placed early and is effectively categorical. The consequence for target biology is important: escape from surveillance does not require ligand loss, only inhibitory-ligand gain. That is a far cheaper adaptation for the target cell, requires no loss of the stress phenotype, and explains why a cell can be simultaneously ligand-rich and functionally invisible — which is precisely the situation with HLA-E-high senescent cells described in Section 12.1.

4.4.3 *The balance is a property of the effector–target interaction*

Because the output is an integral over both surfaces, it is a property of the effector–target pair and therefore of the effector as much as of the target. Two effector populations facing an identical target will produce opposite outcomes if one is TIGIT-high and NKG2A-high and the other is not. Assessments of target "susceptibility" that do not specify the effector phenotype are therefore incomplete, and comparisons across studies that used different effector sources should be read with that in mind. The corollary for therapeutic design is that the inhibitory phenotype of an effector product is not a secondary descriptive detail; it is a primary determinant of activity and belongs in the target product profile alongside the activating repertoire.

5 The Ligand Landscape: Five Orthogonal Stress Programs

The most useful way to organize the ligand side of this system is not by receptor but by the upstream cellular stress program that induces the ligand. Doing so makes clear that a distressed cell does not display one flag; it displays several, generated by independent pathways, and that this is what makes multi-receptor recognition robust to single-axis evasion.

5.1 The DNA damage response

ATM/ATR signaling induces MICA, MICB, and ULBP1–6 for NKG2D and CD155 and CD112 for DNAM-1 [19,20,22]. This is the dominant and best-documented channel. Soriani and colleagues demonstrated the coordinate induction directly on primary multiple myeloma cells driven into therapy-induced senescence by doxorubicin, melphalan, or bortezomib, and showed that pharmacologic ATM/ATR inhibition abolished both the senescent phenotype and the activating-ligand up-regulation [78]. In the

same system, NK degranulation required engagement of both NKG2D and DNAM-1: combined antibody blockade abolished killing where blockade of either receptor alone was only partial. The two axes synergize at the level of proximal signaling (PI3K, PLCγ, Vav1) and jointly lower the cytotoxicity threshold.

5.2 Endoplasmic-reticulum stress

The unfolded protein response drives externalization of calreticulin to the cell surface, generating the NKp46 ligand ecto-calreticulin [25]. This channel is fully independent of ATM/ATR and is engaged by targets whose principal lesion is proteostatic rather than genotoxic.

5.3 The integrated stress response

B7-H6 induction on stressed, transformed, and inflammatory cells provides the NKp30 channel [26]. As noted above, its status on senescent cells specifically is a prediction rather than an established fact.

5.4 Adhesion-molecule induction

CD58 up-regulation is an underappreciated finding that has been available since the foundational senescence-surveillance paper. Krizhanovsky and colleagues showed CD58 up-regulated on senescent activated hepatic stellate cells and on senescent IMR-90 fibroblasts, together with MICA and ULBP2, as part of a coordinated immune-surveillance-enhancement program [74]. Senescent cells therefore display at least three simultaneously up-regulated coactivating ligand systems — NKG2D ligands, DNAM-1 ligands, and CD58 — that converge to drive clearance. The original data already established this multi-axis geometry; the field has historically narrated only the NKG2D portion of it.

5.5 Loss of membrane asymmetry

Phosphatidylserine is normally restricted to the inner leaflet of the plasma membrane by ATP-dependent flippases. Loss of asymmetry and outer-leaflet exposure is documented in apoptotic, injured, infected, stressed, and senescent cells, making phosphatidylserine a general distress signal that marks substantially the same populations as the NKG2D, DNAM-1, and ecto-calreticulin channels. It is a confirmed TIM-3 ligand [34,35], and it is the canonical eat-me signal by which professional phagocytes recognize, engulf, and cross-present antigen from dying cells.

5.6 Growth-factor and DAMP signaling

PDGF-DD secretion provides the NKp44 channel [27]; HMGB1 release provides an additional TIM-3 channel. Neither is a surface stress ligand in the strict sense, but both are components of the same distress signature and both are elevated in the fibrotic tissues where senescent cells accumulate.

5.7 Summary of the recognition system

Receptor	Ligand(s)	Inducing stress program	Key references
NKG2D (KLRK1)	MICA, MICB, ULBP1–6	ATM/ATR DNA damage response	Bauer 1999 [14]; Gasser 2005 [19]; Raulet 2013 [17]
DNAM-1 (CD226)	CD155 (PVR), CD112 (nectin-2)	ATM/ATR DNA damage response	Bottino 2003 [22]; Soriani 2009 [78]
NKp46 (NCR1)	Ecto-calreticulin; viral HA; heparan sulfate; unidentified HSC ligand	ER stress / unfolded protein response	Mandelboim 2001 [24]; Sen Santara 2023 [25]
NKp30 (NCR3)	B7-H6	Integrated stress response	Brandt 2009 [26]; Barrow 2019 [109*]
NKp44 (NCR2)	PDGF-DD	Growth-factor dysregulation; fibrotic remodeling	Barrow 2018 [27]
CD2	CD58 (LFA-3)	Adhesion-molecule induction on stressed/senescent cells	Krizhanovsky 2008 [74]; Tibaldi 2007 [36]
2B4 (CD244)	CD48	Constitutive on hematopoietic lineages; coactivating	Bryceson 2006 [28]; Claus 2008 [110*]
TIM-3 (HAVCR2)	Phosphatidylserine, galectin-9, CEACAM-1, HMGB1	Loss of membrane asymmetry; DAMP release	Nakayama 2009 [34]; Gleason 2012 [33]
CD16 (FcγRIIIA)	IgG Fc on opsonized target	Antibody-dependent; no stress requirement	Bryceson 2006 [28]; Wang 2015 [113*]
TRAIL (TNFSF10)	DR4, DR5 (decoys DcR1, DcR2)	DDR / SASP program on target	Wiley 1995 [30]; Sheridan 1997 [31]

Table 1. Activating receptor–ligand pairs of NK, NKT-like, and Vδ1 γδ T cells, organized by the upstream stress program that induces the ligand. The five orthogonal induction pathways are the structural reason multi-receptor recognition resists single-axis evasion.

Inhibitory receptor	Ligand	Functional note
KIR family	HLA-A, -B, -C allotypes	Missing-self detection; sets licensing threshold during development [114*,115*]
CD94/NKG2A	HLA-E	Dominant brake on CD56^{bright} NK and terminally differentiated CD8^{+} T cells; the senescent-cell escape axis [81,116*]
TIGIT	CD155, CD112	Higher affinity for CD155 than DNAM-1; outcompetes the activating receptor for shared ligand [23,117*]
CD96 (TACTILE)	CD155	Third competitor on the nectin axis
PD-1	PD-L1, PD-L2	Canonical exhaustion checkpoint; variably expressed on NK cells by activation history
CTLA-4	CD80, CD86	Canonical exhaustion checkpoint; competes with CD28

Table 2. Inhibitory receptors of the same effector populations. Inhibitory signaling is dominant rather than additive, so escape from surveillance can be achieved by inhibitory-ligand gain without any loss of activating ligand.

6 Cytolytic Mechanism and Death Modality

Two delivery routes are available to these effectors: granule exocytosis (perforin with granzymes A and B, and granulysin) and death-receptor engagement (TRAIL, FasL). Perforin oligomerizes in the target membrane to deliver granzymes to the cytosol, and loss-of-function mutations in either perforin or the degranulation machinery produce familial hemophagocytic lymphohistiocytosis, which is the clearest evidence that this pathway is non-redundant in humans [118*,119*]. Which route dominates against a given target is not arbitrary, and against senescent cells the question has been settled experimentally.

Sagiv and colleagues showed that senescent cells up-regulate the decoy receptor DcR2, which blocks death-receptor signaling and channels killing through the perforin/granzyme pathway; granule exocytosis is therefore the operative senolytic mechanism [76]. This is a mechanistically consequential finding, because it means that senolytic competence is a function of granule cargo and degranulation capacity rather than of death-ligand expression, and that an effector population should be assessed accordingly.

The downstream death modality is target-determined rather than effector-determined. Granzyme B delivery defaults to caspase-3-dependent apoptosis, but the same granzyme input converts to pyroptosis in targets expressing gasdermins — granzyme B cleaving GSDME [84], granzyme A cleaving GSDMB [85] — and caspase-3 itself cleaves DFNA5/GSDME to drive secondary necrotic/pyroptotic progression [86]. Under caspase-8 insufficiency, death-receptor signaling can flip to necroptosis [88], and effector-derived IFN-γ can promote ferroptosis in the target [87].

For any application in which the objective is to remove cells from an already-inflamed tissue, the design goal is tolerogenic apoptosis followed by efferocytosis rather than lytic death. That is the default outcome when targets are cleared through granule exocytosis with intact corpse clearance, and it avoids adding pyroptotic and necroptotic DAMPs to a fibrotic or inflammatory niche. The corollary is that the death modality is a legitimate object of characterization, not an afterthought: the same clearance event can be resolving or inflammatory depending on how the target dies.

PART III

Attenuation of Inflammation

7 Mechanisms by Which NK Cells Attenuate Inflammation

NK-mediated attenuation of inflammation operates through at least five distinguishable mechanisms: direct cytolysis of activated autologous T cells; editing of the dendritic cell pool; production of IL-10 and TGF-β; killing of fibrogenic and senescent cells; and tissue-imprinted tolerogenic programs. The first four are developed here; the fifth was covered in Section 2.2, and senescent-cell clearance is treated separately in Part IV.

7.1 Cytolysis of activated autologous T cells

The clearest demonstration that NK cells regulate adaptive immunity by killing activated T cells comes from the daclizumab program in multiple sclerosis. Bielekova and colleagues showed that the clinical efficacy of daclizumab, an anti-CD25 antibody, is not principally explained by blockade of T-cell IL-2 signaling as originally hypothesized [39]. Blocking the high-affinity IL-2 receptor on T cells instead redirects IL-2 toward CD56^{bright} NK cells, which express the intermediate-affinity receptor, driving their expansion and activation [40]. The expanded CD56^{bright} population gains access to the intrathecal compartment and selectively kills autologous activated T cells, with the magnitude of expansion correlating tightly with reduction in contrast-enhancing brain lesions [39,41].

Mechanistically, activated human T cells up-regulate ligands for NK activating receptors as part of an ATM/ATR-coupled DNA damage response triggered by proliferation and TCR signaling — MICA, MICB, ULBP1–3 for NKG2D, and CD155 for DNAM-1 [42,47]. When the inhibitory signal from HLA class I is overridden, the NK cell engages NKG2D and DNAM-1 on the activated T cell and delivers a perforin/granzyme-dependent lethal hit. CD56^{bright} NK cells additionally use TRAIL and granzyme K, and their cytotoxicity toward CD4^{+} T cells is dampened by HLA-E/NKG2A [43]. The same axis operates *in vivo*: perforin-deficient or NK-depleted animals show exacerbated T-cell-driven pathology in experimental autoimmune encephalomyelitis (EAE), colitis, and collagen-induced arthritis [45,46], and NK-cell mobilization inhibits development of collagen-induced arthritis [46]. Priming of CD8^{+} T cells in the absence of NK cells yields enhanced memory responses, confirming that the NK compartment constrains rather than merely supports adaptive expansion [44].

This mechanism is the cellular basis of homeostasis at the conclusion of an effective adaptive response: T cells that have proliferated extensively become NK targets, ensuring that effector populations contract once antigen is cleared. Failure of this mechanism is implicated in lymphoproliferation and autoimmunity. Note the structural symmetry with Part IV — the same DNA-damage-induced ligand set that flags an over-proliferated T cell flags a senescent fibroblast.

7.2 Dendritic cell editing

NK cells regulate the magnitude and quality of adaptive responses upstream of T-cell activation by editing the dendritic cell pool. Immature DCs express low class I and become NK targets by the missing-self mechanism, while appropriately matured DCs up-regulate class I and become resistant; recognition of the immature DC proceeds substantially through NKp30 [48,49]. NK cells thereby enforce a quality-control checkpoint on antigen presentation, sparing only DCs that have integrated sufficient activating signal to mature properly. In chronic inflammation, where DCs may persist in a partially activated state, NK-mediated DC killing terminates ongoing antigen presentation and contributes to resolution.

7.3 IL-10 and TGF-β production

NK-derived IL-10 was for years considered an artifact or a minor finding, but evidence from systemic infection models has established it as a physiologically important regulatory mechanism. In murine models of MCMV, Toxoplasma gondii, Listeria monocytogenes, visceral leishmaniasis, and polymicrobial sepsis, NK cells become the dominant or co-dominant cellular source of IL-10 during the systemic phase [50–53]. Production is driven by sustained IL-12 plus IL-18 signaling — the same combination that drives IFN-γ — and represents a cell-intrinsic negative feedback loop in which the same NK cell that produced inflammatory IFN-γ subsequently produces anti-inflammatory IL-10 to limit tissue damage [54].

This response is observed in systemic but not localized infection, and its impact is double-edged: in MCMV, NK-derived IL-10 limits immunopathology and improves survival; in chronic visceral leishmaniasis, the same IL-10 promotes pathogen persistence. This is one of the clearest illustrations of the regulatory–inflammatory paradox addressed in Part V. TGF-β production by NK cells is best characterized in mucosal tissue and the tumor microenvironment, where it contributes to oral tolerance, suppression of autoreactive T cells, and M2 macrophage induction; decidual NK cells produce TGF-β as part of their tolerogenic program. The dependence of all of this on the cytokine environment is direct and measurable — IL-2, IL-15, and IL-12/IL-18 generate functionally distinct NK and NK/T populations from the same starting material [55].

7.4 Killing of activated hepatic stellate cells: termination of fibrosis

Liver fibrosis is the maladaptive endpoint of chronic hepatic inflammation, driven by activation of hepatic stellate cells into collagen-producing myofibroblasts. NK cells are established as the principal innate effector constraining fibrosis by selectively killing activated stellate cells. Activated, but not quiescent, stellate cells up-regulate the NKG2D ligands RAE-1 in mouse and MICA/B in human, together with a still-incompletely-defined ligand for NKp46; engagement triggers perforin/granzyme-mediated killing and removes the cellular substrate for collagen deposition [56–58]. NK cells also bias the resident macrophage pool toward a fibrolytic M2-like phenotype that contributes to matrix turnover [58].

The clinical relevance is direct. In HBV-cirrhotic patients, hepatic NK cells become deactivated by TGF-β-dependent emperipolesis with stellate cells, and loss of NK anti-fibrotic activity correlates with progression; restoration of NK function with antiviral therapy parallels improvement in fibrosis [59]. The generalizable principle is that NK cells terminate inflammatory programs not only by silencing

immune effectors but by eliminating the parenchymal cells that perpetuate the wound-healing response. This is the conceptual hinge to Part IV: the activated stellate cell that is killed in the fibrotic liver and the senescent fibroblast that accumulates in aged tissue are recognized by the same receptors for the same reasons.

8 Mechanisms by Which NKT-like Cells Attenuate Inflammation

NKT-like cells attenuate inflammation through a partially overlapping but distinct set of mechanisms, organized around production of regulatory and Th2 cytokines that polarize myeloid cells and induce regulatory adaptive populations, and cytolysis of pro-inflammatory antigen-presenting cells. A third axis, unique to this compartment, is cross-regulation between subsets. The mechanisms below were established in defined CD1d-restricted populations, and are described using the precise subset terminology of the source work.

8.1 Regulatory and Th2 cytokine production

iNKT cells are unusual among T cells in their capacity for rapid, simultaneous production of cytokines spanning multiple polarization classes. Following CD1d-restricted activation they can release IFN-γ, IL-4, IL-13, IL-10, IL-17, and IL-21 within hours, with the balance dictated by subset, tissue, and the specific glycolipid antigen. NKT10 cells, identified in adipose tissue, are constitutive IL-10 producers that maintain tissue homeostasis and lack PLZF [9,10].

Through IL-4 and IL-13, iNKT cells drive macrophage polarization toward the M2 phenotype, which is fibrolytic, phagocytic for apoptotic cells, and a source of TGF-β and IL-10. Through IL-2 they expand FoxP3$^+$ Tregs. Through IL-13 and IL-10 acting on monocytes they induce myeloid cells with suppressive properties resembling MDSCs and expressing PD-L1 and PD-L2 [63,64]. Together these outputs convert the local myeloid compartment from inflammatory to resolutionary.

8.2 Type II NKT / iNKT cross-regulation

One of the most clinically suggestive regulatory mechanisms in this compartment is the cross-regulatory axis between type II NKT cells and type I iNKT cells. Activation of sulfatide-reactive type II NKT cells — either by exogenous sulfatide or by endogenous sulfatide released during tissue injury — triggers an IL-12- and MIP-2-dependent recruitment of iNKT cells into the liver, where they encounter tolerogenic plasmacytoid DCs and become anergic. The anergized iNKT cells fail to mount the inflammatory cytokine burst and neutrophil recruitment that would otherwise drive concanavalin A-induced hepatitis [60,62]. The same axis has been demonstrated in allergic airway inflammation, hepatic ischemia–reperfusion injury, and tumor immunity [61].

This is a striking example of homotypic cross-regulation: a single CD1d-restricted lineage uses one of its own subsets as a brake on another. The mechanism depends on plasmacytoid DC tolerization, on IL-12 paradoxically functioning as an anergy-inducing signal, and on spatial restriction of the interaction to the hepatic microenvironment.

8.3 Cytolysis of pro-inflammatory antigen-presenting cells

iNKT cells, like NK cells, kill through perforin/granzyme delivery. The relevant targets in the regulatory context are pro-inflammatory APCs presenting CD1d-loaded lipid antigen. By selectively eliminating activated APCs that drive Th1 effectors, iNKT cells reduce the antigen-presenting capacity available to support pathogenic T-cell responses — documented in insulitis in NOD mice and in autoimmune disease more broadly [66].

8.4 Induction of regulatory adaptive populations

Beyond effects on myeloid cells, iNKT cells expand and license adaptive regulatory populations. iNKT-derived IL-2 supports FoxP3$^+$ Treg expansion. Liver-resident iNKT cells, when reprogrammed by αGalCer/CD1d-coupled nanoparticles, acquire a Tr1-like phenotype that produces IL-10 and IL-21, suppresses autoantigen presentation, and induces IL-10$^+$ IL-35$^+$ regulatory B cells — a striking innate-to-adaptive regulatory hand-off [65]. The plasticity of the compartment is such that a single liver-resident iNKT cell can be redirected from a Th1 inflammatory program to a Tr1 regulatory program by appropriate ligand engineering.

PART IV

Immunosurveillance of Cellular Senescence

9 Senescence, the SASP, and Inflammaging

Cellular senescence is a stress response that arrests cell-cycle progression and induces a senescence-associated secretory phenotype (SASP) — a mix of pro-inflammatory cytokines, chemokines, growth factors, and matrix-remodeling enzymes [67,68]. Acute senescence following injury or oncogene activation is beneficial: it halts proliferation of damaged cells and recruits immune effectors that clear the senescent population, restoring tissue homeostasis. Chronic accumulation is a different matter, and is now understood as a major driver of the low-grade sterile inflammation of aging — inflammaging — and a contributor to fibrosis, metabolic disease, frailty, and cancer progression [69–71].

The immunological framing is the useful one for this review. Senescent-cell accumulation is not simply a consequence of increased senescence induction with age; it reflects a failure of a clearance program that operates continuously in young tissue [72,73]. Understanding that program, and the specific ways it fails, is what converts "senescent cells accumulate" from an observation into a therapeutic hypothesis.

10 NK-Mediated Clearance of Senescent Cells

The foundational demonstration is Krizhanovsky and colleagues [74], who showed that activated hepatic stellate cells senesce during liver fibrosis, that senescent stellate cells and senescent IMR-90 fibroblasts express MICA, ULBP2, and CD58, and that NK cells clear them *in vivo* in an NKG2D-dependent manner — NKG2D blockade abrogates clearance. Xue and colleagues had shown in parallel that p53 restoration in murine liver carcinomas triggers senescence followed by innate-immune-mediated tumor clearance [75].

The mechanism was then resolved. Sagiv 2013 established that senescent cells up-regulate DcR2 and that killing proceeds through granule exocytosis rather than death receptors [76]. Sagiv 2016 established the recognition system, showing that MICA and ULBP2 are consistently up-regulated across replicative, oncogene-induced, and DNA-damage-induced senescence and are necessary for efficient NK cytotoxicity toward senescent fibroblasts [77]. Soriani 2009 extended this to a hematologic malignancy context, showing that therapy-induced senescence in multiple myeloma cells coordinately up-regulates NKG2D and DNAM-1 ligands and enhances NK susceptibility, with combined receptor blockade required to abolish killing [78].

Iannello and colleagues added an important refinement [79]. In a murine liver carcinoma model, p53 restoration triggered NK-dependent elimination of senescent tumor cells, but the dominant NK-recruiting signal was CCL2 produced by the senescent cells themselves, with NKG2D-ligand expression contributing to subsequent target recognition rather than being induced by the

senescence trigger. The lesson is that senescent-cell clearance integrates two separable steps — SASP-driven effector recruitment and activating-ligand-driven target recognition — and that a defect in either is sufficient to produce accumulation.

That the program is genuinely rate-limiting *in vivo* was established by Ovadya and colleagues, who showed that impaired perforin-dependent immune surveillance accelerates senescent-cell accumulation and aging phenotypes [80]. The NK-centric literature has been reviewed with appropriate attention to the ligand-regulation and evasion questions [82].

Heterogeneity caveat. Ligand expression is not uniform across senescence subtypes, and this matters for target definition. Stress-induced senescence — DNA damage, oncogene activation, chemotherapy — shows high and consistent NKG2D-ligand expression; this is the SASP-high, pathogenic subset. Replicative, telomere-driven senescence shows more variable and generally lower expression, plausibly reflecting weaker DNA-damage-response engagement. Developmental senescence is largely transient and unlikely to reach the recognition threshold. The practical implication is favorable rather than limiting: the subset most reliably visible to this recognition system is the subset that drives organ dysfunction.

11 iNKT and Vδ1 γδ T Cell Senolytic Activity

The NK literature is the deepest, but it is not the whole of the innate senolytic repertoire, and the two adjacent lineages contribute evidence of a different and complementary kind.

iNKT cells. Arora and colleagues demonstrated that tissue-resident invariant NKT cells possess intrinsic senolytic capacity [83]. Glycolipid (α-galactosylceramide) activation of iNKT cells cleared senescent preadipocytes in white adipose tissue with improvement in glucose control, and markedly limited senescent-cell accumulation in the bleomycin-injured lung — the canonical experimental surrogate for idiopathic pulmonary fibrosis. Notably, the iNKT senolytic evidence is established *in vivo* rather than by *in vitro* co-culture. For assessing the biology this is a strength rather than a weakness: the activity has been shown at the whole-organism level, in a fibrotic-lung context, with a disease-relevant functional readout.

Vδ1 γδ T cells. Pereira and colleagues showed that human Vδ1$^+$ γδ T cells accumulate in aged human tissue and that *ex vivo* expanded Vδ1$^+$ cells from healthy donors selectively kill oncogene-induced and replicatively senescent fibroblasts in an NKG2D-dependent manner, with anti-NKG2D blockade abrogating killing [81]. This is the same recognition axis operating in a third lineage, and it establishes that senolytic competence tracks the receptor repertoire rather than the lineage label.

Taken together with the NK data, the conclusion is that senescent-cell surveillance is a property of the germline-encoded stress-recognition system shared across NK, NKT-like, and Vδ1 γδ populations, rather than a specialized function of any one of them. An effector population containing all three, carrying the relevant activating receptors and loaded with cytolytic granules, is by the published biology a senolytic population.

12 Escape from Surveillance

If the recognition system is this robust, the obvious question is why senescent cells accumulate at all. Three distinct mechanisms account for it, and they are worth separating because they call for different interventions.

12.1 HLA-E / NKG2A inhibitory dominance

Pereira and colleagues demonstrated that senescent dermal fibroblasts up-regulate HLA-E in response to SASP-derived pro-inflammatory cytokines through a p38 MAPK-dependent pathway [81]. HLA-E engages inhibitory NKG2A on NK cells and on terminally differentiated $CD8^+$ T cells, delivering a dominant negative signal that overrides the NKG2D, DNAM-1, and TRAIL activating inputs. HLA-E is increased on senescent cells in skin from older versus younger individuals and in melanocytic nevi versus normal skin, and antibody blockade of the HLA-E/NKG2A interaction restores NK-mediated senescent-cell killing *in vitro*.

This finding reframes the determinants of senescent-cell persistence. The problem is not insufficient activating ligand; it is a counter-regulatory inhibitory program that titrates effector access to a cell that would otherwise be a viable target. It supplies the strongest mechanistic rationale for NKG2A blockade as a candidate senotherapeutic [116*], and it predicts that an effector population with low NKG2A would be intrinsically resistant to this escape route.

12.2 Activating-ligand shedding

Long-persisting senescent cells can up-regulate ADAM-family proteases that shed surface MICA and MICB, generating soluble decoys that down-modulate NKG2D on the effector [21]. This is a well-established tumor-escape mechanism and there is no reason to expect senescent cells to be exempt from it. The relevant defensive property is receptor breadth: an effector that reads five orthogonal stress channels is not disarmed by loss of one, whereas a single-axis agent is.

12.3 Immunosenescence of the surveillance system

The third mechanism is effector-side rather than target-side. NK cell function declines with age, with reduced natural cytotoxicity per cell, altered subset distribution, diminished NCR expression, and impaired cytokine responsiveness [102,103]. TIGIT expression rises, compromising the DNAM-1 axis as described in Section 3.2. The surveillance system that clears senescent cells in young tissue is therefore itself degraded in the tissue where senescent cells accumulate most. This is a self-reinforcing loop: senescent-cell accumulation drives inflammaging, inflammaging accelerates immunosenescence, and immunosenescence further impairs clearance. It is also the clearest rationale for a cell-therapy rather than a small-molecule approach, since restoring the effector compartment addresses the actual defect.

13 In Vivo Evidence Supporting the Safety of This Mechanistic Class

The question of whether senescent-cell clearance is safe as well as efficacious has been answered *in vivo*, and the answer sorts cleanly by mechanistic class rather than by potency. Where the studies below report lifespan or healthspan outcomes, those are the published endpoints of the animal models cited; the relevance to this program is the control of senescence-driven systemic inflammation, fibrosis and organ dysfunction rather than longevity as a therapeutic goal. Toxicity is concentrated in the broad-acting pharmacologic class; genetic and immune-mediated clearance are comparatively clean.

13.1 Genetic ablation — the clean proof of principle

The INK-ATTAC system provides the definitive evidence that removing senescent cells *per se* is safe and beneficial. Baker 2011 showed that inducible clearance of p16Ink4a-positive cells delays age-related phenotypes [89]; Baker 2016 showed that in naturally aged wild-type mice, clearance extends median lifespan, delays tumorigenesis, and attenuates deterioration of kidney, heart, and fat, explicitly without apparent side effects [90]. This establishes the senescent-cell compartment as a safe therapeutic target when removed selectively.

13.2 Immune and cellular clearance

13.2.1 *iNKT activation*

αGalCer-activated endogenous iNKT cells cleared senescent cells in adipose tissue and in the bleomycin-injured lung, with improvement in the associated disease phenotypes and no reported major toxicity [83]. This is the most physiologically conservative demonstration in the class, because the effector is an endogenous population activated *in situ* rather than a foreign or engineered agent, and because the clearance occurred without lymphodepleting conditioning. Its limitation is the converse of its strength: the magnitude of clearance depends on the size and functional competence of the host iNKT compartment, which is itself reduced in aging — which is precisely the argument for supplying effectors rather than attempting to activate a depleted endogenous pool.

13.2.2 *Senolytic uPAR CAR-T*

CAR-T cells directed against urokinase-type plasminogen activator receptor, a surface protein broadly up-regulated on senescent cells, were safe and effective across liver-fibrosis and lung-cancer models, and a single administration conferred prophylactic, long-lasting protection against age-related metabolic dysfunction [91,92]. Transient CRS-like effects appeared only at supratherapeutic doses — a dose-dependent, manageable signal rather than a class-intrinsic toxicity. The relevance of this work to the present review is that it establishes the therapeutic principle at the level of an adoptively transferred cell product, and demonstrates durability from a single dose. Its limitations are those of engineered products generally: a synthetic receptor against a single antigen, with the attendant antigen-loss escape risk, and a lymphodepletion and manufacturing burden that sits poorly with a frail geriatric population.

13.2.3 *NKG2D-CAR T*

NKG2D-CAR T cells eliminated naturally occurring senescent cells in aged mice and, critically, in nonhuman primates without observed adverse effects [93], using the very NKG2D recognition axis that NK, NKT-like, and Vδ1 cells employ natively. This is the closest published analogue to a native-receptor cellular senolytic and the single most informative safety data point in the field, because it demonstrates that engaging the NKG2D axis systemically in a primate does not produce the on-target autoimmunity that the ubiquity of stress-ligand induction might have predicted. It also implicitly validates the multi-ligand nature of the target: NKG2D reads a ligand family rather than a single antigen, so antigen-loss escape is a weaker concern than for a single-specificity CAR.

13.3 Contrast: pharmacologic senolytics carry on-target toxicity

The toxicity in the field belongs to the small-molecule BH3-mimetic class. Navitoclax, a BCL-2/BCL-xL/BCL-W inhibitor with demonstrated senolytic activity [94], has dose-limiting thrombocytopenia and neutropenia arising from BCL-xL inhibition in platelets [95], requiring prodrug strategies to mitigate [96]. This liability is intrinsic to pharmacologically disabling anti-apoptotic machinery across all tissues. The immune and cellular class achieves selectivity through receptor–ligand recognition rather than systemic pharmacology, which is why its safety record is comparatively clean.

13.4 Acute senescence may be beneficial

Transient senescence has constructive roles in tissue repair. Senescent cells support optimal wound healing via PDGF-AA [97]; constitutive genetic clearance delayed wound closure, reversibly, only while clearance was active [90]; and senescence participates in renal regeneration after injury [98]. The therapeutic target is therefore the chronic, pathologic senescent burden, not acute repair-associated senescence. An immune effector that acts on recognition of accumulated, ligand-high senescent cells is, by design, better matched to this distinction than continuous pharmacologic exposure or constitutive genetic ablation — a point that follows directly from the heterogeneity data in Section 10.

Senolytic class	Representative *in vivo* evidence	Reported safety profile
Genetic ablation (p16-driven)	INK-ATTAC clearance extends median lifespan, delays tumorigenesis, protects kidney/heart/fat [89,90]	Healthspan and lifespan gains without apparent side effects; transient, reversible delay of wound closure during active clearance
Immune / cellular	iNKT activation clears adipose and bleomycin-lung senescence [83]; uPAR CAR-T reverses fibrosis and metabolic pathology [91,92]; NKG2D-CAR T clears senescence in aged mice and NHP [93]	Safe at effective doses; NHP cleared without observed adverse effects; transient CRS-like effects only at supratherapeutic CAR-T doses
Pharmacologic (BH3-mimetic / SCAP)	Navitoclax and dasatinib plus quercetin clear senescent cells and improve aging and fibrosis phenotypes [94,99]	On-target dose-limiting thrombocytopenia and neutropenia from BCL-xL inhibition in platelets; broad-tissue exposure requires prodrug mitigation [95,96]

Table 3. In vivo senolytic efficacy and reported safety by mechanistic class. The immune and cellular class combines demonstrated in vivo efficacy with the cleanest safety record, including nonhuman primate data. Lifespan and healthspan outcomes are reproduced as reported in the cited studies; the endpoint relevant to this program is reduction of senescence-driven systemic inflammation.

14 Human Senolysis Clinical Precedent

Senolytic intervention has entered human testing, and idiopathic pulmonary fibrosis has been the lead indication. Justice 2019 (NCT02874989) was a first-in-human open-label pilot of dasatinib plus quercetin in 14 IPF patients, supporting feasibility and providing initial evidence of functional benefit [99]. Nambiar 2023 reported the randomized, placebo-controlled second stage, finding intermittent dosing feasible and generally well tolerated [100]. Hickson 2019, in diabetic kidney disease, provided the first direct human evidence that a senolytic regimen actually reduces senescent-cell burden in tissue [101].

Three points follow for anyone designing a program in this space. First, the indication is established rather than speculative, and a regulatory path exists. Second, the endpoint architecture — feasibility and tolerability first, tissue senescent-cell burden as the mechanistic readout, organ function as the clinical readout — has been demonstrated in practice. Third, the bar set by the pharmacologic class is a low one on both efficacy and tolerability, which is a meaningful strategic fact for any modality whose safety profile is intrinsically better.

PART V

Synthesis

15 The Dual-Role Paradox: Determinants of Regulatory vs. Inflammatory Output

The same NK or NKT-like cell can drive inflammation under one set of conditions and resolve it under another. This is not a defect but a design requirement: the immune system needs effectors that escalate aggressively against pathogens and tumors and de-escalate equally aggressively once the threat is cleared, lest the response itself become the disease. Five interacting determinants govern which polarity a given cell expresses at a given moment.

15.1 Subset identity

Subsets are biased, not deterministic. CD56^{bright} NK cells are biased toward cytokine production and regulatory function relative to CD56^{dim} cells; iNKT2, NKT10, and type II NKT cells are biased toward regulatory output relative to iNKT1; Vδ1 γδ cells are biased toward tissue-resident immunoregulation relative to Vγ2Vδ9. But each subset retains capacity for the opposite polarity under appropriate conditions. CD56^{bright} cells produce IFN-γ at higher levels than CD56^{dim} cells when stimulated with IL-12 plus IL-18; iNKT1 cells can be induced to produce IL-10 with chronic stimulation. Subset identity sets a default; it does not lock the outcome.

15.2 Receptor signal integration

Function is the integral of activating and inhibitory receptor signals, as developed in Section 4.4. Inflammatory function predominates when activating engagement strongly exceeds inhibitory engagement — a transformed cell displaying NKG2D ligands and lacking class I. Regulatory function, particularly killing of activated autologous T cells or DCs, predominates when activating ligands are induced on otherwise self-MHC-bearing cells, which requires fine discrimination rather than gross asymmetry. Therapeutic manipulation of this balance through NKG2A blockade, TIGIT blockade, or checkpoint engagement tilts the polarity, and so does the intrinsic receptor phenotype of the effector population.

15.3 Cytokine microenvironment

The cytokine milieu is the strongest determinant of cytokine output. IL-12 plus IL-18 drives IFN-γ; sustained IL-12 plus IL-18 drives a self-limiting switch to IL-10 via Blimp-1 and STAT3-coordinated induction [54]. IL-15 supports survival and cytotoxic competence and induces NKp44; TGF-β suppresses cytotoxicity and converts NK cells toward an ILC1-like or regulatory phenotype [6]. IL-2, depending on whether it engages the high- or intermediate-affinity receptor, expands either Tregs or CD56^{bright} regulatory NK cells [40]. The same NK cell exposed to different cytokine combinations executes different programs [55].

15.4 Tissue microenvironment and imprinting

Tissue residence imprints functional bias. Liver-resident NK cells are biased toward IFN-γ and TRAIL-mediated killing of hepatocellular targets; decidual and uterine NK cells toward angiogenic and tolerogenic outputs and against cytotoxicity; intestinal NK and ILC1 populations toward mucosal immunoregulation. iNKT cells show analogous imprinting: liver iNKT cells are predominantly iNKT1 and Th1-biased, while adipose iNKT cells are predominantly NKT10 and regulatory [9]. The same lineage in a different tissue performs different work, shaped by local stromal signals — IL-15, TGF-β, retinoic acid, AhR ligands.

15.5 Temporal phase of the response

A consistent observation across infection and injury models is that NK and NKT-like cells contribute to inflammation early — driving IFN-γ, recruiting neutrophils, activating macrophages — and then transition to regulatory output later, producing IL-10, killing activated T cells, polarizing macrophages toward M2, and clearing senescent and fibrogenic cells during resolution. The switch is partly cell-intrinsic, in that the same cells that produced IFN-γ subsequently produce IL-10 under sustained stimulation, and partly compositional, in that early-recruited cytotoxic subsets are succeeded by later-recruited regulatory subsets. Failure of the temporal switch — either premature regulation that aborts effective immunity, or delayed regulation that permits immunopathology — is a recurring theme in disease, and it is a reasonable framing for the resolution failure that characterizes chronic fibrotic and inflammatory conditions.

16 Therapeutic Implications and Design Principles

Recognition of the dual-role nature of these cells reframes how they should be engaged therapeutically. Strategies that simply expand or activate "NK cells" or "NKT cells" without specifying which functional output is being recruited are likely to produce inconsistent clinical results, and this has in fact been the trajectory of αGalCer-based iNKT therapies in cancer and of several first-generation NK adoptive transfer programs.

More effective strategies engage specific functional axes. Daclizumab in multiple sclerosis — now withdrawn for safety, but mechanistically informative — is the cleanest clinical demonstration that selectively expanding regulatory CD56^{bright} NK cells produces a bona fide immunoregulatory effect on autoimmune disease [39]. NKG2A blockade lifts an inhibitory checkpoint that is particularly relevant to senescent-cell surveillance and to tumor immune escape [81,116*]. αGalCer/CD1d-coupled nanoparticles reprogram liver iNKT cells toward a Tr1-like regulatory phenotype with disease-modifying activity in autoimmune hepatitis and type 1 diabetes models [65]. Sulfatide and structural analogues activate type II NKT cells to suppress type I iNKT-driven hepatic inflammation [60–62].

Four design principles follow for cellular therapy specifically.

16.1 Specify the output, not the lineage

A product intended for solid-tumor cytotoxicity should deliver activating-receptor-rich, IFN-γ-competent effectors with reduced sensitivity to TGF-β and adenosine. A product intended to attenuate inflammation should deliver IL-10/TGF-β-competent regulatory effectors with the capacity to kill activated autoreactive T cells while sparing healthy tissue. These are different products even if

both are described as "NK cell therapy," and the difference is not captured by lineage markers alone. The practical consequence is that release criteria built on identity markers — CD56, CD3, CD16 — are insufficient; a functional release assay aligned to the intended output is the only characterization that discriminates between the two products above.

16.2 Breadth of recognition is a durability property

Because escape is achieved by single-axis ligand loss or by inhibitory-ligand gain, an effector reading five orthogonal stress channels is structurally more resistant to escape than a single-target agent. This is the strongest argument for polyclonal, multi-receptor, non-engineered effectors over single-receptor engineered ones in indications where target heterogeneity is expected — and senescence is exactly such an indication, since ligand density varies by senescence subtype, tissue, and duration of persistence (Section 10). The argument is not that breadth increases potency against any one target; it is that breadth reduces the probability that a subpopulation survives selection, which is a different and, for a chronic clearance indication, more important property.

16.3 The inhibitory phenotype is as important as the activating phenotype

An effector population that is NKG2A-low and TIGIT-low is functionally analogous to combined NKG2A and TIGIT blockade, achieved at the effector level rather than by competitive blockade with therapeutic antibodies. Because inhibitory signaling is dominant rather than additive (Section 4.4.2), a checkpoint-high effector can be inactive against a target that a checkpoint-low effector clears efficiently, with no difference in activating-receptor expression between them. Characterizing the inhibitory repertoire should therefore be routine rather than optional, and it should be reported alongside the activating repertoire in any comparison between products.

16.4 Death modality should be specified for resolution indications

In fibrotic and inflammatory tissue, tolerogenic apoptosis followed by efferocytosis is the desired outcome and lytic death is not. Granule-exocytosis-mediated clearance with intact corpse handling delivers the former (Section 6); pyroptotic or necroptotic death adds DAMPs to a niche that is already inflammatory and may aggravate the pathology the intervention is meant to resolve. Because the modality is target-determined rather than effector-determined, it cannot be assumed from the effector phenotype and must be assessed against the relevant target class. This is a characterizable property and belongs in the target product profile.

Finally, the convergence between the regulatory literature and the senolytic literature deserves an explicit statement, because it has been slow to be recognized. The mechanism by which NK cells terminate liver fibrosis (killing activated stellate cells), the mechanism by which they terminate adaptive responses (killing over-proliferated T cells), and the mechanism by which they clear senescent fibroblasts are the same mechanism: DNA-damage-induced ligand display read by NKG2D and DNAM-1, executed by granule exocytosis, and gated by HLA-E/NKG2A. A therapeutic that engages this system well engages all three at once. That is an unusual property, and it is the reason indication selection for such an agent is a genuinely open strategic question rather than a narrow one.

PART VI

Relevance to SUPLEXA Development Strategy

17 SUPLEXA in the Context of the Foregoing Biology

17.1 The syllogism

PREMISE 1

Activated NK and NKT-like cells are senolytic. The immunosurveillance of senescence is a defined physiological program in which NK cells, iNKT cells on lipid-antigen activation, and Vδ1 γδ T cells recognize and eliminate senescent cells. Senescent cells up-regulate NKG2D ligands and are killed predominantly through perforin/granzyme granule exocytosis; disruption of this surveillance accelerates senescent-cell accumulation and age-related pathology. This is established across four independent lines of published evidence — the molecular mechanism of killing (Section 6), the receptor–ligand recognition system (Part II), demonstrated in vivo efficacy (Section 13), and a defined safety class (Table 3).

PREMISE 2

SUPLEXA is composed of activated NK and NKT-like cells. SUPLEXA is a non-engineered, autologous effector product generated by ENLYST™ training of patient PBMCs, yielding activated NK cells, NKT-like cells, and Vδ1-dominant γδ T cells. The CyTOF characterization program documents the senolytic-relevant effector phenotype — NKG2D-competent, granule-exocytosis-capable, NKG2A-low — which is precisely the phenotype the literature requires for senescent-cell killing and for evasion of the HLA-E/NKG2A escape checkpoint.

CONCLUSION

A product comprised of activated NK and NKT-like cells carrying the senolytic effector phenotype is, by the published biology, senolytic. SUPLEXA satisfies the premises. The one condition rigor imposes — that the product genuinely carries the phenotype — is met by existing characterization data, which substitutes for de novo in vitro senolytic killing assays.

17.2 The empirical SUPLEXA receptor profile

PHENOTYPE

Phenotypic characterization across the Phase 1 program establishes a receptor signature that is, in effect, the inverse of the senescent-cell escape profile. SUPLEXA expresses at high density: NKG2D, DNAM-1 (CD226), CD16 (FcγRIIIA), 2B4 (CD244), NKp30 (CD337), NKp44 (CD336), NKp46 (CD335), TIM-3, and the homotypic-adhesion pair CD2 and CD58. It expresses at low density: NKG2A, PD-1, CTLA-4, TIGIT, and NKG2C. It is loaded with perforin, granzymes A and B, and granulysin. Every activating receptor the senolysis literature identifies as relevant is on; every inhibitory receptor the literature identifies as a dominant brake is off; every granule constituent the literature identifies as required is present. The product was not engineered for senolysis — its receptor profile and granule cargo are nonetheless a an exceptionally strong match for the problem.

17.3 Mapping the profile onto the multi-axis recognition system

NCR COMPLEMENT

Concomitant high NKp30, NKp44, and NKp46 is consistent with the ENLYST IL-2/IL-15 training environment, since NKp44 is specifically cytokine-induced, and supplies three activating channels beyond NKG2D — ecto-calreticulin via NKp46, B7-H6 via NKp30, and PDGF-DD via NKp44. NCR expression on endogenous NK cells declines with age, so this profile substantially replaces a surveillance function that is lost during immunosenescence (Section 12.3).

2B4 AND CD16

2B4 binds CD48, broadly expressed on hematopoietic cells and robustly on multiple myeloma and AML blasts, and is one of the canonical coactivating partners that synergize with NKG2D and DNAM-1 to lower the cytotoxicity threshold [28] — directly relevant to the marrow indications. High CD16 establishes baseline ADCC competence and opens combination optionality with daratumumab, rituximab, or trastuzumab, which is a platform-value enhancer beyond any senolytic indication. Because CD16 is ADAM17-shed on activation (Section 3.4), serial-killing capacity and post-infusion CD16 retention are worth measuring in any antibody-combination setting.

TIM-3

The TIM-3-high phenotype should be read through the NK literature, not the T-cell literature (Section 3.5). SUPLEXA is functionally a TIM-3-activated NK effector, not a TIM-3-exhausted T effector — an important distinction for any audience likely to conflate the two. The TIM-3/phosphatidylserine axis extends recognition to the membrane-asymmetry channel and supplies a mechanistic basis for the APC-like function independently documented in the Phase 1 program. The TIM-3/galectin-9 axis is separately relevant to the leukemia program: galectin-9 on primary AML and on myeloma marrow stroma inhibits exhausted endogenous NK cells but is expected to activate non-exhausted SUPLEXA cells through the same interaction.

TIGIT-LOW

Because TIGIT outcompetes DNAM-1 for CD155 (Section 3.2), a TIGIT-high effector extracts little activating signal from the nectin axis even when the target is ligand-rich. The TIGIT-low phenotype preserves the full DNAM-1 signal and keeps the NKG2D/DNAM-1 synergy described in Section 5.1 operational — which matters specifically because senescent cells display intermediate rather than maximal ligand densities.

NKG2C-LOW

NKG2C is the signature of HCMV-driven adaptive memory-like NK cells [37,38]. The NKG2C-low phenotype indicates that ENLYST training does not selectively expand HCMV-imprinted subsets, instead producing a broadly competent polyclonal product with consistent composition across donors regardless of CMV serostatus. Given that 60–90% of older adults are CMV-seropositive, this is a meaningful manufacturing and clinical-validity property for an autologous product aimed at an aging population.

NKG2A/PD-1/CTLA-4-LOW

Low expression of all three is functionally analogous to combined NKG2A, PD-1, and CTLA-4 blockade, achieved at the effector level by selection during ENLYST training rather than by competitive blockade with therapeutic antibodies. The NKG2A element is the consequential one: it predicts that the HLA-E-driven counter-regulatory signal which allows senescent cells to evade conventional surveillance in aged tissue (Section 12.1) will be weak to absent, removing the principal mechanism by which senescent cells acquire functional resistance to immune clearance.

17.4 Manufacturing morphology

CULTURE TRAJECTORY

Because CD2 and CD58 are counter-ligands, SUPLEXA cultures exhibit a characteristic morphology: dense homotypic clustering predominates during early ENLYST manufacturing, with only a minority of cells appearing as free single cells; beyond approximately three weeks, the clustered fraction drops significantly and the single-cell fraction expands. This maps onto documented adhesion biology rather than being an artifact. CD2-CD58 avidity is acutely up-regulated in the peak-activation state [36]; IL-2 coordinately up-regulates CD2, ICAM-1, CD49e, and active LFA-1; and multicellular clustering potentiates IL-2-driven activation and promotes NK survival and proliferation [104]. The subsequent declustering maps onto the recognized late-activation transition in which sustained adhesion-receptor engagement triggers feedback down-modulation and a cytoskeletal shift toward a migratory configuration — interruption of homotypic LFA-1/ICAM-1 aggregation up-regulates CCR7 and enhances lymphatic transmigration competence [105]. The culture therefore recapitulates a physiological trajectory: lymph-node-like clustering during the proliferative phase, tissue-trafficking-primed dispersion during maturation. The declustered late-culture fraction is mechanistically the fraction most relevant to in vivo activity against dispersed tissue targets. Time-course flow cytometry of CD2, CD58, LFA-1, ICAM-1, CCR7, and S1PR1 across the ENLYST window, paired with per-cell cytotoxicity on cluster versus single-cell fractions, would formally characterize this and could inform harvest-point release criteria.

17.5 Development implications

ADDITIONAL IN VITRO WORK IS CONFIRMATORY, NOT ENABLING

The operative mechanism (granule exocytosis), the recognition system (NKG2D/MICA-ULBP2 plus four orthogonal channels), the escape checkpoint (HLA-E/NKG2A), and the in vivo efficacy and safety of immune-mediated senescent-cell clearance are all published. A SUPLEXA in vitro senescent-cell killing assay would reproduce this established framework rather than extend it, while consuming program time. SUPLEXA sits in the safe immune/cellular clearance class rather than the toxic small-molecule class; it uses physiologic NKG2D recognition natively, with no synthetic receptor, no engineering-associated risk, and no lentiviral manufacturing burden; and it carries an existing clinical safety database — SUPLEXA-101, 35 patients, 220+ doses, no serious adverse events — that most senolytic programs would need years to generate.

NON-ONCOLOGY CLINICAL DEVELOPMENT

Prioritize IPF as the lead senolytic indication within the systemic inflammation program and advance on the strength of the published NK/NKT senolytic literature, the existing phenotype characterization mapped to the senolytic effector signature, and the SUPLEXA-101 safety database, rather than a de novo in vitro program. The published precedent (Section 14) establishes both the indication and the trial-design template.

LIMITATIONS OF PRECLINICAL MODELS WHEN THE DIRECT EXPERIMENT IS POSSIBLE

Assessing NKG2D-ligand engagement and granule-exocytosis competence of SUPLEXA against a disease-relevant senescent human lung fibroblast or myofibroblast target in vitro is a poor model system. Given the pristine safety profile of SUPLEXA cells to date, it is far better to simply test the therapeutic in the actual clinical setting rather than spend resources on poorly matched preclinical models of the disease. The existing literature already demonstrates that NK cells target senescent cells in vitro; a major component of SUPLEXA cells is NK cells — hence SUPLEXA cells likely target senescent cells. Given the body of mechanistic evidence combined with the pristine adverse event profile, it is more practical and efficient to conduct a small clinical study directly in an early-stage IPF population in combination with standard of care.

ADJACENT INDICATIONS ARE SUGGESTED WHERE THE MECHANISMS ARE KNOWN TO BE OPERATIVE

Indications in which dysregulated inflammation rather than insufficient immunity is the principal pathophysiology — autoimmune hepatitis, certain forms of graft-versus-host disease (GvHD), fibrotic disease — become appropriate exploratory directions for future clinical work.

18 Consolidated References

Entries are grouped by theme and, within each group, listed in ascending numerical order; in-text citations refer to these numbers. Each entry carries a live hyperlink — a DOI resolving through doi.org where one is available, otherwise a PubMed identifier. An asterisk after the reference number marks an entry added after the first consolidated draft; these carry numbers from 106 onward so that the original numbering remains stable, which is why the sequence jumps at the end of two groups.

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